# A MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF TWO SPECIES OF POSADASIA

## P. CAPSULATA (DARLING) MOORE AND P. PYRIFORMIS MOORE

MORRIS MOORE

*Mycologist to The Barnard Free Skin and Cancer Hospital, St. Louis*

*Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany of Washington University*

### INTRODUCTION

The etiological agent of histoplasmosis has for a number of years been incorrectly determined. The disease, made well-known in a series of papers by the original describer of the infection, Darling ('06, '08, '09), was attributed to a protozoon. Later investigators considered it a *Cryptococcus* (da Rocha-Lima, '12, '13; Castellani and Chalmers, '19; Vuillemin, '31) or an *Oidium*. This confusion may have been due to the inability of the earlier workers to grow the organism, all systematic classification being based on smears or tissue sections. The present author, upon receipt of an organism from a proven case of histoplasmosis, sought to determine the correct position of the fungus.

The disease itself is a serious and apparently fatal infectious condition which is present in America and resembles kala-azar of India and Tropical America. It is characterized clinically by emaciation, severe anemia, with a marked leucopenia, splenomegaly, enlargement of the liver, and irregular pyrexia. Pathologically, the organism is found to invade the endothelial cells in the smaller lymph and blood vessels and capillaries. The affected organs become necrosed and the liver develops cirrhosis. The lungs and small and large intestines are studded with pseudotubercles, giving the appearance of miliary tuberculosis. The peribronchial lymph nodes usually are enlarged, showing some tubercles most of which become ulcerated.

Issued May 25, 1935.

The first case that came under Darling's observation was that of a negro, a native of Martinique, 27 years of age, who was mildly delirious and incoherent in his speech. The patient showed an enlarged spleen. The autopsy picture was typical of what has been pointed out previously. The second case was also that of a Martiniquan negro. The third case was a Chinese shopkeeper, who had lived on or near the Isthmus of Panama for 15 years.

The organism of *Posadasia capsulata,* studied in this paper, was received from Charles Thom, Principal Mycologist of the United States Department of Agriculture. It had been sent to him by DeMonbreun of Vanderbilt University, who isolated it from a case of histoplasmosis, described by Dodd and Tompkins ('34). DeMonbreun ('34) apparently proved the pathogenicity of the organism by carrying it through experimental animals and recovering the pathogen. Dodd and Tompkins ('33) and DeMonbreun ('33) reported their findings at the meeting of the Society for Tropical Medicine at Richmond, Virginia, November 15, 1933.

The second organism, *P. pyriformis,* was received from G. H. Hansmann, formerly of Iowa City and now of Georgetown University School of Medicine. Hansmann and Schenken ('33) had reported a case before the American Association of Pathologists and Bacteriologists at their Washington meeting on May 9, 1933. The patient was a white male, 43 years old at time of death. He had had a refractory skin ailment for the last 16 years of his life. This was a scaly inflammation underneath which the skin was thick and red, becoming more intense during 1929. In June of 1932 the skin showed hard nodules, and in July the patient developed a high fever, signs of pleurisy, and died on August 7. The microbe was cultured from dermal elevated spots and an enlarged lymph node. The fungus was considered an *Oidium.*

## TECHNIQUE

Hanging-drop, water mounts, and also simple water mounts on a slide revealed the general outline of the organism clearly. Mounts made with Amann's lacto-phenol also gave favorable

results, but those in crystal violet in glycerine were only fair. The mycelium of these fungi is such that only the large cells and the asci show any indication of cellular construction. The hyphae are fairly small, and the details are obscured by the wall, which is quite thick here.

In order to trace the development of a single ascus, beef-extract gelatine mounts were made with a suspension of mycelium, gelatine being used in place of agar because of its greater transparency. Many of the cell interpretations are based on a study of sectioned materials.

## Description

The life cycles of yeast-like fungi are usually complicated, because different habitats change the type of growth. In many cases, a different mycelium is linked with the loss of infectivity, or the change from a parasitic to a saprophytic mode of life. In any case, the organisms involved here existed as simple yeast-like cells, spherical or ovoid, varying from 1 to 4 $\mu$ in long axis, usually 3 $\mu$. According to Darling, these cells are surrounded by a clear refractile and non-staining rim which equals in thickness about one-sixth the total diameter of the cell. The structure of these cells is found to be non-homogeneous, having a granular protoplasm which may be vacuolated. These cells multiply by elongation and fission according to da Rocha-Lima ('12, '13), which led him to consider these fungi as members of the genus *Cryptococcus*.

When transferred to an artificial substrate, the organism changes to a mycelial growth with aerial and submerged hyphae. When kept in blood and serum cultures at 37° C., however, and transferred at short intervals the yeast-like form persists or predominates, according to observations by DeMonbreun ('34) and also by Thom. All studies made here are from cultures which had already formed mycelium, having no evidence of yeast-like cells.

The filamentation consists of multi-celled hyphae 1–5 $\mu$ in diameter, with various types of morphology (pls. 11–14). There finally results a tubercled ascus containing a number of small spores. The yeast-like cells thus undergo a state of

elongation which gives them a sclerotic appearance, to form filaments.

To describe the later development of the fungus on an artificial substrate, it seems best to start with the ascospore which supposedly is the germ of the colony. These spores (pl. 11, fig. 1; pl. 13, fig. 72) are small, and when set free from the ascus germinate to form a mycelium such as is shown in the illustrations. The hyphae are multi-celled and, from indications of cytological work in progress, coenocytic. These cells may be elongated and narrow (pl. 11, fig. 2) or short and thick, usually depending on the type of medium. The filaments may develop peculiar swellings (pl. 11, figs. 32, 38, 42; pl. 12, fig. 55; pl. 13, figs. 75, 82, 90), or they may give rise to the well-known racquet mycelium (pl. 11, figs. 4, 8, 16–17; pl. 13, figs. 77, 97). The hyphae may further be distorted and present peculiar types of development (pl. 11, figs. 40, 47; pl. 13, fig. 101).

In addition, there are a number of types of cells which play an important rôle both in propagation and conservation of the organism. These organs include chlamydospores, conidia, and asci. The chlamydospores vary in size and proportions in *Posadasia capsulata* and *P. pyriformis*. They may be intercalary, 3–10 $\mu$ in diameter, and may occur singly (pl. 11, fig. 23) or in groups or chains (pl. 13, fig. 96) and may also be found laterally (pl. 11, fig. 30; pl. 12, fig. 64; pl. 14, fig. 109), sessile or pedicellate. They may also occur terminally as enlarged forms, 3–10 × 6–20 $\mu$. On serum agar in the moist region of the colony are series of cells (pl. 13, fig. 95) which simulate chlamydospores but are actually yeast-like.

Conidia are cells of great importance in propagation. They occur laterally, are sessile or pedicellate, spherical or pyriform, 2–8 $\mu$ in diameter (pl. 11, figs. 23, 28; pl. 12, figs. 60, 64). They are present on most media, especially on substrates with a low pH. On serum agar in *P. capsulata* they are found at just above or just under the surface, while in *P. pyriformis* they are mostly above the surface. Conidia break off easily from the hyphae and germinate (pl. 11, fig. 1; pl. 13, fig. 72) to give rise to a new colony serving much the same purpose as do the ascospores.

In all the observations of the microbes presented here, no indication of sexuality has made itself evident. Instead, the non-sexual ascus develops terminally, laterally, or as an intercalary organ. Asci form first as globose or clavate cells on short or long pedicels or on one- to several-celled filaments. These cells enlarge and may become 5–18 $\mu$ in diameter or in long axis. The pedicels may be perpendicular to the hyphae (pl. 14, figs. 106–107). The presumptive asci have thick walls (pl. 11, figs. 35–36; pl. 12, fig. 53) and when placed in a water mount show numerous oil globules, representing a large food-storage supply. In *P. capsulata* these cells are spherical or become so to form the spherical ascus typical of the species, while in *P. pyriformis* they may be spherical or pyriform. When young the presumptive asci are smooth-surfaced and thick-walled. As they grow older those present at or on the surface of the medium become somewhat pitted, then spinose (pl. 12, figs. 56, 65; pl. 14, figs. 113–115), while those below the surface or submerged in the agar remain smooth. The spines develop into variously shaped tubercles in the adult ascus. In the case of *P. capsulata,* on some of the substrates there are more asci at or on the surface than within the medium, whereas in *P. pyriformis* these cells form a heavy and deep layer on the surface with smaller smooth forms just below the surface.

The tubercles of the asci, mentioned above, vary in number and size and may become long finger-like projections simulating germ-tubes (pl. 12, fig. 66). These may also be spherical emergences (pl. 11, fig. 51; pl. 12, figs. 58, 67; pl. 14, fig. 122) or short blunt outgrowths (pl. 12, figs. 62–63, 68–70), or they may be situated almost diametrically opposite as large blunt membranous growths (pl. 12, figs. 57, 61). In most cases, however, there are combinations of nearly all types, particularly long and short tubercles. These are comparatively smaller on the pyriform asci in *P. pyriformis* (pl. 14, figs. 118, 125, 130). The so-called germ-tubes may vary from approximately .5 to 7 $\mu$ in the adult ascus. The asci themselves, exclusive of the tubercles, vary in size in both species. The spherical forms vary from 5 to 25 $\mu$ in diameter, while the pyriform asci, present only in *P. pyriformis,* are 6–12 $\times$ 12–26 $\mu$, usually 10 $\times$ 22 $\mu$.

Cytological investigations show that the single ascus contains a number of spherical spores which are set free by a rupture of the ascus wall to germinate and commence another cycle. When in a nutrient condition the ascus may germinate with few to several germ-tubes which develop into a mycelium (pl. 12, fig. 71). Apparently, the tubercles play no part in reproduction or propagation, for a study of their structure shows them to be clear and void of any protoplasm.

## CULTURAL DESCRIPTIONS

The cultures obtained were transferred to Sabouraud's glucose agar. Since, as previously emphasized, a study of an organism should not be limited to a single substrate, a number of standard media were inoculated and grown at room temperature, approximately 22° C. It was desirable to make a comparative study of both organisms, and like media were therefore seeded with the two species. The following artificial substrates are arranged in the order of their decreasing hydrogen-ion concentration.

### POSADASIA CAPSULATA

*Raulin's Agar (pH 4.1).*—Growth poor, diameter approximately 2 cm. after 43 days. Colony white and cottony. Mycelium variable in size and shape; hyphae 2–3 $\mu$ in diameter, with intercalary chlamydospores approximately 10 $\mu$ in diameter; racquet mycelium; terminal hypnospores 9 × 18 $\mu$; large round cells at end of a thin filament approximately 10 $\mu$ in diameter; conidia 3–5 $\mu$ in diameter; tuberculate asci lacking.

*Richards' Agar (pH 4.3).*—Colony approximately 2.5 cm. in diameter after 43 days. Point of inoculation somewhat heaped up, white and cottony. Mycelium mostly submerged in the agar. Culture consists chiefly of long branching hyphae 2–3 $\mu$ in diameter; many small round cells approximately 3 $\mu$ in diameter, large round cells terminal on long thin filaments, 5–10 $\mu$ in diameter; conidia ovoid or pyriform, 5 $\mu$ in diameter; intercalary chlamydospores 5–10 $\mu$ in diameter, terminal hypnospores as above; racquet mycelium present; asci with tubercles not apparent.

*Czapek's Agar (pH 4.4).*—Colony approximately 3 cm. in diameter after 43 days. Growth loose and cottony, color white. Mycelium similar to that found on the above two media.

*Malt Extract Agar (Product of Digestive Ferments Co., pH 4.6).*—Colony approximately 4.2 cm. in diameter after 43 days. Development of ridges from point of inoculation to woolly periphery. Color light Isabella. Abundant mycelium of hyphae 2–3 $\mu$ in diameter; racquet mycelium; conidia 3–5 $\mu$ in diameter, spherical on short pedicels, or pyriform; intercalary chlamydospores 5–8 $\mu$ in diameter, singly or in chains; terminal clavate and elongate cells varying in size and shape with the age of the organism; many small round cells approximately 3 $\mu$ in diameter, probably conidia; asci numerous, tuberculate, 15–20 $\mu$; round cells on long thin filaments, approximately 1.5 $\mu$ in diameter, probably young asci.

*Wort Agar (Product of Digestive Ferments Co., pH 4.8).*—Colony approximately 3 cm. in diameter after 43 days. Growth thick and cottony. Color light Isabella. Racquet mycelium abundant, with hyphae approximately 3 $\mu$ in diameter; intercalary chlamydospores 5–8 $\mu$ in diameter; terminal spherical cells 5–10 $\mu$ in diameter; conidia numerous, 5 $\mu$ in diameter; many asci 10–20 $\mu$ with tubercles or club-like projections up to approximately 7 $\mu$ in length.

*Sabouraud's Agar (pH 5.6).*—Colony approximately 4.7 cm. in diameter after 43 days. Growth cottony, showing clumps similar to pleomorphic changes found in cultures of dermatophytes, color light Isabella. Hyphae 2–3 $\mu$ in diameter; conidia 3–5 $\mu$ in diameter, mostly spherical; intercalary chlamydospores spherical, 5–7 $\mu$ in diameter; racquet mycelium present; asci numerous, 15–18 $\mu$ in diameter, with many tubercles projecting from the surface of the ascus.

*Sabouraud's Broth (The above minus the agar, pH 5.6).*—Growth of flakes or clumps, chiefly at bottom of flask. Surface shows a folded growth, cottony, with upright hyphae on surface macroscopically similar to columellae. Mycelium on surface white to light Isabella in color, similar to that found on agar. Broth becoming dark with growth of organism. Submerged growth of long branching hyphae 1.5–2 $\mu$ in diameter.

Clavate cells terminal on filaments, varying in proportion and size.

*Corn-Meal Agar (Product of Digestive Ferments Co., pH 6.0).*—Colony cottony, slightly heavy at point of inoculation, white in color, with a diameter of approximately 1.5 cm. after 43 days. Hyphae 2–3 $\mu$ in diameter, showing many swellings; racquet mycelium very much enlarged; conidia thick-walled, approximately 5 $\mu$ in diameter; intercalary chlamydospores approximately 8 $\mu$ in diameter; terminal hypnospores also present; terminal clavate cells approximately 10 $\mu$ in diameter; asci 10–15 $\mu$ in diameter.

*Potato-Dextrose Agar (pH 6.2).*—Colony very much heaped-up in center, cottony, white to light Isabella in color, 3 cm. in diameter after 43 days. Cells larger than on the above media. Hyphae 3–4 $\mu$ in diameter, some larger; racquet mycelium very characteristic, swollen portions much enlarged; conidia 5–7 $\mu$ in diameter; intercalary chlamydospores 8–10 $\mu$ in diameter, spherical lateral forms on 2- or 3-celled pedicels, 6 $\mu$ in diameter, many occurring singly as well as in chains; clavate sessile cells; asci larger than others, up to 25 $\mu$ in diameter.

*Lactose Broth (Product of Digestive Ferments Co., pH 6.8).*—Flakes of cottony growth submerged in the medium with surface growth. Mycelium of long branching hyphae approximately 2 $\mu$ in diameter; very few swollen cells or racquet formation; very few conidia; terminal clavate cells present; tuberculate asci absent in submerged mycelium. Aerial growth similar to that found on an agar substrate of the same constituents.

*Lactose Agar (The above plus 2 per cent agar).*—Colony cottony, white to light Isabella in color, showing radiating ridges from the inoculum, attaining a diameter of approximately 3 cm. after 43 days. Hyphae small, many 1–1.5 $\mu$ in diameter, some 2–3 $\mu$; conidia numerous, 3–5 $\mu$ in diameter; racquet mycelium present; intercalary chlamydospores 5–8 $\mu$ in diameter; asci approximately 15 $\mu$ in diameter.

*Nutrient Agar (Product of Digestive Ferments Co., pH 7.0).*—Growth fair, colony sparsely cottony, somewhat flat, with a diameter of approximately 2.8 cm. after 43 days. Color light Isabella. Hyphae 2–3 $\mu$ in diameter; conidia sessile and pedi-

cellate, approximately 5 $\mu$ in diameter; racquet mycelium present; clavate terminal cells and intercalary chlamydospores as on lactose agar; asci as large as 22 $\mu$ in diameter with tubercles.

*Glycerine Agar (Beef-extract agar plus 6 per cent glycerine, pH 7.2).*—Thick cottony growth with an elevated inoculum approximately 2.5 cm. in diameter after 43 days. Color light Isabella. Hyphae 2–3 $\mu$ in diameter; sclerotic cells present; many conidia, spherical and pyriform, 3–5 $\mu$ in diameter; terminal clavate cells, spherical cells on long thin filaments, 5–10 $\mu$ in diameter; chlamydospores 5–8 $\mu$ in diameter; asci approximately 15 $\mu$ in diameter; many hyphal swellings.

*Serum Agar (Bacto-beef blood serum, product of Digestive Ferments Co., plus 2 per cent agar, pH 7.3).*—Colony approximately 2.5 cm. in diameter after 43 days, with deep radiating ridges from a somewhat white cottony point of inoculation to a moist and flat periphery. Hyphae tend to be thick and heavier and appear coremioid. Mycelium generally thicker, with the various cells present; conidia spherical and pyriform, sessile or pedicellate, 5 $\mu$ in diameter; chlamydospores approximately 8 $\mu$ in diameter; many large round cells in chains; asci up to 15 $\mu$ in diameter, plus the tubercles.

*Endo's Agar (Product of Digestive Ferments Co., pH 7.5).*—Culture assumes the pink color from the medium, cottony in appearance, with a diameter of approximately 3 cm. after 43 days. Microscopic appearance similar to that on nutrient agar.

### POSADASIA PYRIFORMIS

*Raulin's Agar (pH 4.1).*—Colony approximately 2 cm. after 43 days. Culture white and cottony at inoculum, submerged in the medium. Mycelium shows racquet formation with swellings; hyphae 2–3 $\mu$ in diameter; conidia sessile or pedicellate, spherical or pyriform, 5–7 $\mu$ in diameter; intercalary chlamydospores spherical or slightly elongated, 3–10 $\mu$ in diameter; clavate or spherical cells on pedicels, 6–10 $\mu$ in diameter; asci below the agar without tubercles, above the surface multi-tuberculate, spherical 6–18 $\mu$, pyriform 8–10 $\times$ 18–26 $\mu$, in general $9 \times 24$ $\mu$.

*Richards' Agar (pH 4.3).*—Colony approximately 2 cm. in diameter after 43 days. Culture cottony, white, and heaped-up at point of inoculation. Hyphae 3–4 $\mu$ in diameter; conidia approximately 5 $\mu$ in diameter; racquet mycelium present; intercalary chlamydospores 5–8 $\mu$ in diameter; clavate cells on long filaments; asci terminal on long several-celled pedicels, or lateral on short pedicels, spherical approximately 12 $\mu$ in diameter, pyriform 8–10 × 16–24 $\mu$.

*Czapek's Agar (pH 4.4).*—Growth loose and cottony, approximately 4.5 cm. in diameter after 43 days. Color white. General characters similar to those on the above media. Hyphae 2–3 $\mu$ in diameter; intercalary chlamydospores 5–7 $\mu$ in diameter.

*Malt Extract Agar (Product of Digestive Ferments Co., pH 4.6).*—Colony approximately 3 cm. in diameter after 43 days. Culture cottony and somewhat flat with a heaped-up center and showing concentric circles of growth, Isabella color. Racquet mycelium in abundance with numerous swellings; hyphae 2–3 $\mu$ in diameter, some larger; conidia sessile or pedicellate, spherical or pyriform, 5–8 $\mu$ in diameter; intercalary chlamydospores 3–8 $\mu$ in diameter; terminal clavate cells present; asci relatively few in number, spherical predominating, approximately 12 $\mu$ in diameter.

*Wort Agar (Product of Digestive Ferments Co., pH 4.8).*—Growth thick and cottony, appearing cerebriform and convolute at point of inoculation. Diameter approximately 2 cm. after 43 days' growth. Color light Isabella. Racquet mycelium abundant; hyphae 2–4 $\mu$ in diameter; intercalary chlamydospores 3–8 $\mu$ in diameter; conidia as on the above medium; mycelium somewhat resembling chains of oidia, with the cells 6–8 × 7–12 $\mu$; spherical asci predominating, approximately 12 $\mu$ in diameter.

*Sabouraud's Agar (pH 5.6).*—Growth thick and cottony, approximately 6 cm. in diameter after 43 days. Colony Isabella-colored in center, approximately 1 cm. high, with a concentric ridge surrounded by a lighter woolly periphery. Hyphae 2–4 $\mu$ in diameter; racquet mycelium present; intercalary

chlamydospores 3–5 $\mu$ in diameter; conidia approximately 5 $\mu$ in diameter, both spherical and pyriform; asci numerous, on surface of agar, spherical 6–22 $\mu$ in diameter, pyriform 6–10 × 12–24 $\mu$.

*Sabouraud's Broth (The above minus the agar).*—Broth has turned dark with increased growth of flakes of mycelium. Surface shows a thick growth of Isabella-colored mycelium similar to that on the agar. Submerged mycelium of long hyphae approximately 2 $\mu$ in diameter with some variations; few swellings or racquet formations; clavate cells reduced in size.

*Corn-Meal Agar (Product of Digestive Ferments Co., pH 6.0).*—Growth poor, colony approximately 1.5 cm. in diameter after 43 days. Culture cottony with an irregular periphery, Isabella-colored. Cells and hyphae thicker, with the filaments 2–5 $\mu$ in diameter; conidia spherical or pyriform, approximately 5 $\mu$ in diameter; racquet mycelium present; spherical asci predominating, 6–22 $\mu$ in diameter; many thick-walled cells.

*Potato-Dextrose Agar (pH 6.2).*—Colony 5 cm. in diameter after 43 days. Culture cottony and somewhat flat with concentric rings of growth, color Isabella. Hyphae 2–4 $\mu$ in diameter; racquet mycelium and hyphal swellings; intercalary chlamydospores 5–7 $\mu$ in diameter; conidia 3–8 $\mu$ in diameter, both pyriform and spherical; many long filaments, approximately 100 $\mu$, bearing a clavate cell terminally, the presumptive ascus; asci both spherical and pyriform, 6–22 $\mu$ in diameter.

*Lactose Broth (Product of Digestive Ferments Co., pH 6.8).*—Growth of submerged flakes of mycelium, with some Isabella-colored lumpy cottony colonies on the surface. Mycelium on surface similar to that on the agar; submerged mycelium of long hyphae approximately 2 $\mu$ in diameter; few hyphal swellings or racquet mycelium; few conidia or intercalary chlamydospores; many spherical and clavate terminal cells; cells much reduced in size.

*Lactose Agar (The above plus 2 per cent agar).*—Colony 3 cm. in diameter after 43 days. Culture cottony and somewhat flat except for a woolly periphery, color Isabella. Hyphae 2–3 $\mu$

in diameter; conidia 3–5 $\mu$ in diameter, both spherical and pyriform; intercalary chlamydospores 3–7 $\mu$ in diameter; racquet mycelium and hyphal swelling present; spherical asci 6–20 $\mu$ in diameter, pyriform 6–12 × 10–22 $\mu$.

*Nutrient Agar (Product of Digestive Ferments Co., pH 7.0).*—Growth similar to that on lactose agar.

*Glycerine Agar (Beef extract agar plus 6 per cent glycerine, pH 7.2).*—Colony approximately 4 cm. in diameter after 43 days. Culture cottony with center showing convolutions and a splitting of the agar. Color Isabella. Cells short and thick; racquet mycelium abundant with hyphal swellings; intercalary chlamydospores 5–8 $\mu$ in diameter; conidia spherical and pyriform, approximately 5 $\mu$ in diameter; spherical cells 8–9 $\mu$ in diameter present in series in a hypha; spherical asci up to 22 $\mu$ in diameter, pyriform 6–10 × 10–24 $\mu$, with well-defined tubercles mostly spherical or blunt.

*Serum Agar (Bacto-beef blood serum, product of Digestive Ferments Co., plus 2 per cent agar, pH 7.3).*—Colony approximately 2.5 cm. in diameter after 43 days. Culture cottony, light Isabella, with a flat moist periphery suggestive of a yeast-like condition. Hyphae 2–3 $\mu$ in diameter; racquet mycelium abundant; conidia sessile or pedicellate, spherical or pyriform, approximately 5 $\mu$ in diameter; terminal subclavate to clavate cells; spherical asci 6–15 $\mu$ in diameter, and pyriform of varying proportions; intercalary chlamydospores 5–7 $\mu$ in diameter.

*Endo's Agar (Product of Digestive Ferments Co., pH 7.5).*—Colony approximately 3 cm. in diameter after 43 days. Culture cottony with the central portion red and a pink coloration spread throughout due to the absorption of the dye in the medium by the mycelium. Characteristics similar to those found on nutrient agar.

*Gelatine.*—No liquefaction by either species, or if present extremely slow.

*Litmus Milk.*—No acid or curdling, but an alkaline reaction which is interpreted as negative.

*Carbohydrate Reactions.*—No fermentation of any sugar used. No acid or gas production.

## Discussion

The causative agent of histoplasmosis, as has been pointed out, has passed through a period of nomenclatorial change, due in all cases to the failure of the organism to grow on an artificial substrate. The original name *Histoplasma capsulatum* was assigned to it by Darling on the basis that it was a protozoon. He described the microbe as small, round or oval, 1–4 $\mu$ in diameter, possessing a polymorphous chromatin nucleus, basophilic cytoplasm, and achromatic spaces all enclosed within an achromatic refractile capsule. In smears, the parasite to him represented certain features found in protozoa by Leishman, Donovan, and several others. Furthermore, he thought he observed some forms possessing flagellae, and consequently he placed the organism in the Flagellidia, or the flagellates as we now know them.

This view was held for a number of years. In 1912 and 1913 da Rocha-Lima published papers dealing with lymphangitis in horses due to *Cryptococcus farciminosus*. He also studied material taken from cases of Darling's histoplasmosis, using the Romanowsky stain. On the basis of the differential staining reaction he concluded that the organism was a fungus of the genus *Cryptococcus* and closely related to *C. farciminosus*. His opinion was based also on the fact that the type of lesion produced simulated that of the above infection. Furthermore, with the Romanowsky dye, protozoa show up with a blue protoplasm within which is a large red clump called the macronucleus and also a smaller clump, the micronucleus or blepharoblast. This latter inclusion is lacking in Darling's organism. In addition, the type of division of the cell was suggestive of *Cryptococcus*. Da Rocha-Lima's idea was further emphasized by Castellani, Neveu-Lemaire (quoted by Vuillemin), and adhered to by most text-books.

The unfortunate circumstance in all this nomenclature was the fact that the organism was not cultured. The problem of naming a microbe of this kind, therefore, depends on the kind of growth obtained on an artificial substrate, in addition to the form in which it exists as a parasite. The morphology of an

organism is usually the first criterion that taxonomists consider in classification. In these two fungi, an association of these facts as described led to placing the two species in the genus *Posadasia* Canton, *Posadasia pyriformis* being a new species, and *P. capsulata* a new combination as published in a previous paper (Moore, '34).

The genus *Posadasia* was created by Canton in 1898 as the representative etiological agent of coccidioidal granuloma of South America. Da Fonseca and Areã Leão ('28) supposedly examined Posada's organism and found that the spores dehisced through the membrane. Such an observation might easily confuse an investigator and lead to an erroneous conclusion. Illustrations seem to show the organism in tissue to be similar to the asci here described on agar, a point further emphasized by Magalhães ('32) who illustrated the organism with tubercles and named it *Neogeotrichum pulmoneum*.

A series of papers by Floriano de Almeida ('33, '33a, '34, '34a) brings out a relationship between the organisms described here and *Coccidioides immitis* and *Paracoccidioides brasiliensis* as found in the tissues of individuals in South America (Brazil). He described particularly the latter, illustrating the typical club formation found in cultures of *Posadasia* and present in *Paracoccidioides brasiliensis*. It is quite evident that these organisms are closely related and that some, such as *Coccidioides immitis* and *Paracoccidioides brasiliensis*, as described by Almeida, have a specialized life cycle (Moore, '32). *Posadasia capsulata* and *P. pyriformis*, on the other hand, show a yeast-like stage in the host, a reduced condition which develops on artificial media to the type of reproductive body identical with that of *Paracoccidioides*.

Because of several observations made by various investigators, the genus *Posadasia* was made synonymous with *Coccidioides* by the author (Moore, '32). However, after examining *Posadasia capsulata* and *P. pyriformis*, and since the work of Almeida, it seems quite apparent that the genus should stand as an entity. It is on this basis that the two above species are established, showing a relationship to *Coccidioides immitis* of the family Coccidioideaceae and its relative *Paracoccidioides*

*brasiliensis* which should constitute a genus of the same family. Further, reviewing all data at hand, it seems that *Posadasia capsulata* and *P. pyriformis* form the connecting link between the family Coccidioideaceae and the family Endomycetaceae of the Endomycetales. These two fungi, therefore, occupy a position in the Coccidioideaceae, closely related to the family Endomycetaceae. On the basis of these views, the family Coccidioideaceae has the following members:

**Coccidioideaceae**
**Posadasia**
*Posadasia capsulata*
*Posadasia pyriformis*
**Coccidioides**
*Coccidioides immitis*
**Paracoccidioides**
*Paracoccidioides brasiliensis*
**Rhinosporidium**
*Rhinosporidium Seeberi*

In recent publications, Redaelli and Ciferri ('34; Ciferri and Redaelli, '34) have created the family Histoplasmaceae and included it with the families Nectaromycetaceae Cif. and Red. and Torulopsidaceae Cif. in the super family Adelosaccharomycetaceae Guilliermond, comprising all the non spore-bearing yeasts. Basing their evidence on the pathology of the host, cultural, morphological and biochemical reactions of the organisms, they place *Cryptococcus farciminosus* Rivolta and Micellone, 1883, and *Cryptococcus muris* Shortt, 1923, in the genus *Histoplasma,* with *H. capsulatum* as the type species. It is difficult to conceive of a relationship existing between these three fungi, and furthermore the presence of asci in *Histoplasma (Posadasia)* eliminates a family classification in the non spore-forming yeasts.

## Summary

1. Two cases of Darling's histoplasmosis yielded two species, *Posadasia capsulata, P. pyriformis.*
2. The organisms were studied on many substrates covering

a fairly wide range of hydrogen-ion concentration and with a varying amount of carbohydrate and nitrogen.

3. *Posadasia capsulata* differs from *P. pyriformis* in its smaller size and amount of growth; spherical tuberculate asci as compared with both spherical and pyriform asci of the latter; white to light Isabella color as compared with Isabella to light cinnamon for the latter.

4. The organism occurs in the host as a small cell 1–4 $\mu$ in diameter. On an artificial substrate a mycelium is developed which is aerial and also submerged in the medium, giving rise to many characteristic cells as chlamydospores, conidia, racquet mycelium. Tuberculate asci develop from globose or clavate cells in the absence of a sexual act.

5. With these two species, the family Coccidioideaceae now has four genera and five species: *Posadasia capsulata, P. pyriformis, Coccidioides immitis, Paracoccidioides brasiliensis,* and *Rhinosporidium Seeberi.*

## ACKNOWLEDGMENTS

The author wishes to express his thanks to Dr. Carroll W. Dodge, Professor of Botany in the Henry Shaw School of Botany of Washington University, and Dr. George T. Moore, Director of the Missouri Botanical Garden, for the courtesies extended; Dr. Charles Thom, Principal Mycologist, United States Department of Agriculture, for the culture of *Posadasia capsulata;* and Dr. George H. Hansmann of Georgetown University, School of Medicine, for the culture of *P. pyriformis.*

## BIBLIOGRAPHY

Almeida, Floriano P. de ('33). Notas sobre a morphologia de "Paracoccidioides brasiliensis" nos tecidos parasitados. Rev. Biol. Hyg. **4**: 4 pp. April 1934.

————————, ('33a). Blastomicoses em geral e sua classificação. Definiçao e classificação das blastomicoses. Rev. Assoc. Paul. Med. **3**: 270–277. 1933.

————————, ('34). Le blastomicosi nel Brasile. Fol. Clin. Biol. **6**[1]: 15 pp. 1934.

————————, ('34a). Formaçoes radiadas de membrana dos cogumelos parasitos. Ann. Facul. Med. Univ. d. Paulo **10**: 163–174. *7 pl. 14 figs.* 1934.

Castellani, A., and A. J. Chalmers ('19). Manual of tropical medicine. pp. 1669–1670. William Wood & Co., New York, 3rd ed. 1919.

Ciferri, R., and P. Redaelli ('34). Sulla posizione sistematica dell' agente patogeno del farcino equino. Boll. Ist. Sier. Mil. **10**: Oct. 1934.

Da Fonseca, O., et A. E. Arêa Leão ('28). Sur la granulome coccidioidal. Formes d'évolution du parasite dans les tissus, dans le pus des ganglions lymphatiques et dans les cultures. Position systematique de *Coccidioides immitis*. Compt. Rend. Soc. Biol. **98**: 619–621. 1928.

Da Rocha-Lima, H. ('12). Histoplasmosis und epizootische Lymphangitis. Arch. f. Schiffs- u. Tropenhyg. Beih. **16**: 79–85. 1912.

————————, ('13). Beitrag zur Kenntnis der Blastomykosen. Lymphangitis epizootica und Histoplasmosis. Centralbl. f. Bakt. I. **67**: 233–249. 1913.

Darling, S. T. ('06). A protozoon general infection producing pseudotubercles in the lungs and focal necroses in the liver, spleen and lymph nodes. Am. Med. Assoc., Jour. **46**: 1283–1285. 1906.

————————, ('08). Histoplasmosis: A fatal infectious disease resembling kala-azar found among natives of Tropical America. Arch. Int. Med. **11**: 107–123. 1908.

————————, ('09). The morphology of the parasite *(Histoplasma capsulatum)* and the lesions of histoplasmosis. A fatal disease of Tropical America. Jour. Exp. Med. & Hyg. **11**: 515–531. 1909.

DeMonbreun, W. A. ('33). Cultivation of the micro-organism of Darling's histoplasmosis. [title only.] South. Med. Jour. **26**: 1005. 1933.

————————, ('34). The cultivation and cultural characteristics of Darling's *Histoplasma capsulatum*. Amer. Jour. Trop. Med. **14**: 93–126. 1934.

Dodd, K., and E. H. Tompkins ('33). A case of Darling's histoplasmosis (so-called Cryptococcus infection) in an infant. [title only.] South. Med. Jour. **26**: 1005. 1933.

————————, ('34). A case of histoplasmosis of Darling in an infant. Amer. Jour. Trop. Med. **14**: 127–138. 1934.

Hansmann, G. H., and J. R. Schenken ('33). New disease caused by a yeast-like organism. Science **77**: 460. 1933.

Magalhães, O. de ('32). A classificação do *"Oidium brasiliense" Neogeotrichum pulmoneum*, n.g. (O. Magalhães, 1914), emend. O. Magalhães, 1931. Inst. Oswaldo Cruz, Mem. **26**: 151–168. 1932.

Moore, M. ('32). Coccidioidal granuloma: A classification of the causative agent, *Coccidioides immitis*. Ann. Mo. Bot. Gard. **19**: 397–428. 1932.

————————, ('34). *Posadasia pyriformis* and *P. capsulata*, two causative organisms of Darling's histoplasmosis in the United States. *Ibid.* **21**: 347–348. 1934.

Redaelli, P., and R. Ciferri ('34). Affinité entre les agents de l'histoplasmose humaine, du farcin equin et d'une mycose spontanée des muridées. Boll. Sez. Ital. Soc. Int. Micro. **10**: Oct. 1934.

Vuillemin, P. ('31). Les champignons parasites et les mycoses de l'homme. Encyclopédie mycologique. 291 pp. Lechevalier. Paris. 1931.

## EXPLANATION OF PLATE

### PLATE 11

*Posadasia capsulata*

All figures drawn as correctly as possible with the aid of a camera lucida at a magnification of × 1440 and reduced to × 750.

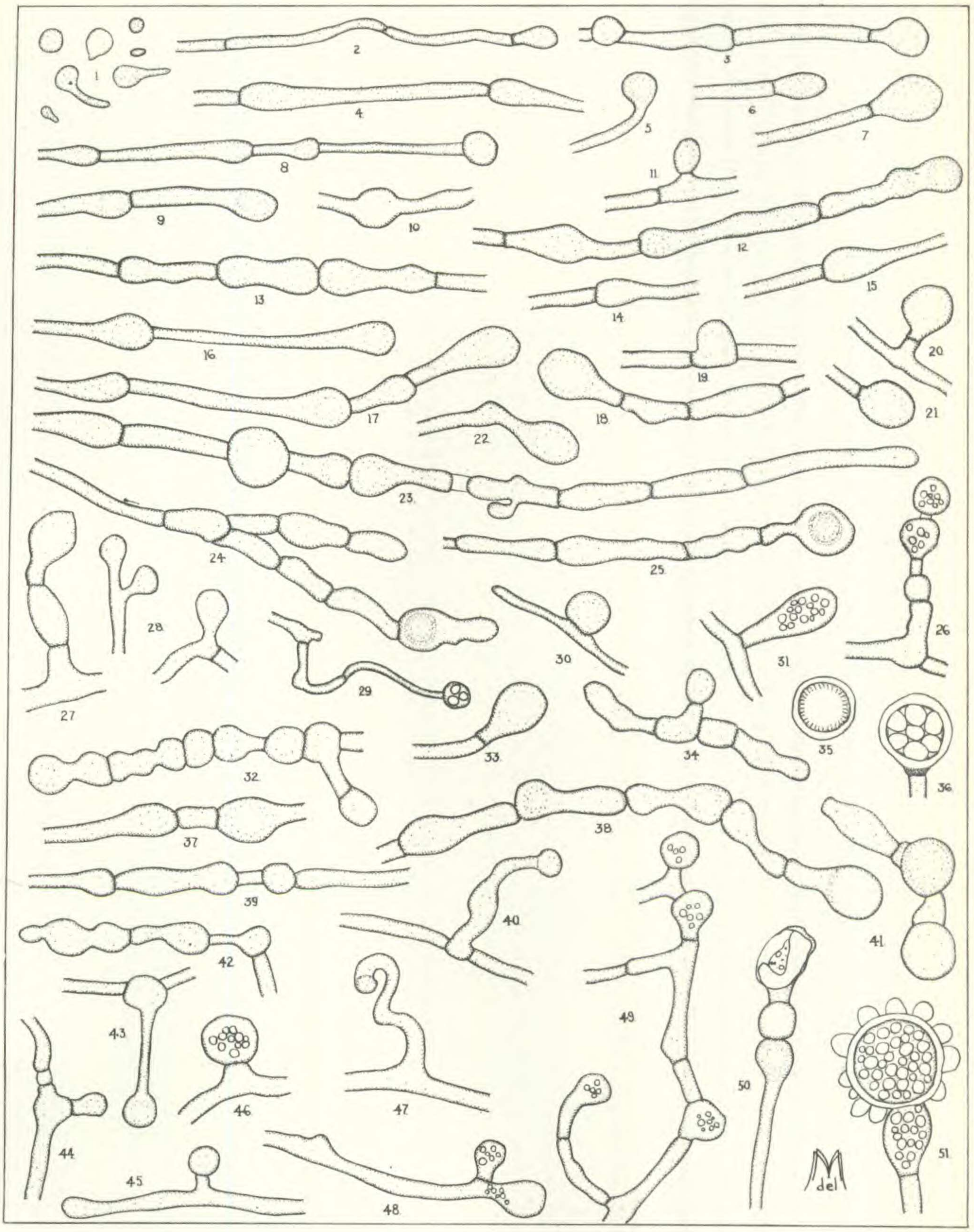
Fig. 1. Group of germinating spores and conidia on lactose agar.
Fig. 2. Hypha with clavate terminal cell on Sabouraud's agar.
Fig. 3. Mycelium on Raulin's agar.
Fig. 4. Racquet mycelium on Sabouraud's agar.
Fig. 5. Terminal clavate cell on wort agar.
Fig. 6. Terminal clavate cell on potato-dextrose agar.
Fig. 7. Terminal clavate cell on corn-meal agar.
Fig. 8. Racquet mycelium with terminal spherical cell on malt extract agar.
Fig. 9. Racquet mycelium on wort agar.
Fig. 10. Spherical hyphal enlargement on malt extract agar.
Fig. 11. Blastosporoid cell on Sabouraud's agar.
Fig. 12. Type of mycelium on malt extract agar.
Fig. 13. Hyphal swellings on Raulin's agar.
Fig. 14. Racquet formation on lactose agar.
Fig. 15. Racquet formation on corn-meal agar.
Fig. 16. Racquet formation on serum agar.
Fig. 17. Racquet formation on serum agar.
Fig. 18. Mycelium on Raulin's agar.
Fig. 19. Chlamydospore on wort agar.
Fig. 20. Lateral clavate cell, presumptive ascus on potato-dextrose agar.
Fig. 21. Terminal clavate cell, as fig. 20 on wort agar.
Fig. 22. Young terminal clavate swelling on potato-dextrose agar.
Figs. 23–25. Mycelium on Raulin's agar showing swellings, chlamydospores, and conidia.
Fig. 26. Young spherical cells, showing oil globules on potato-dextrose agar.
Fig. 27. Presumptive ascus on glycerine agar.
Fig. 28. Conidia on corn-meal agar.
Fig. 29. Young cell on thin filament, possibly a young ascus on Sabouraud's agar.
Fig. 30. Lateral thick-walled resting cell on corn-meal agar.
Fig. 31. Lateral clavate cell with oil globules on potato-dextrose agar.
Fig. 32. Type of mycelium on malt extract agar.
Fig. 33. Possible terminal hypnospore on wort agar.
Fig. 34. Blastosporoid cell on corn-meal agar.
Fig. 35. Spherical cell in lacto-phenol on Richards' agar.
Fig. 36. Young ascus in lacto-phenol, showing internal network on wort agar.
Fig. 37. Mycelium on wort agar.
Fig. 38. Mycelium on Raulin's agar.
Fig. 39. Mycelium on lactose agar.
Fig. 40. Lateral outgrowth which will probably develop an ascus on glycerine agar.
Fig. 41. Hyphal swellings on potato-dextrose agar.

## EXPLANATION OF PLATE

### PLATE 11 *(Continued)*

*Posadasia capsulata*

Fig. 42. Hyphal swellings on corn-meal agar.
Fig. 43. Young globose cell on lateral filament, on malt extract agar.
Fig. 44. Lateral pyriform conidium on potato-dextrose agar.
Fig. 45. Lateral spherical conidium on malt extract agar.
Fig. 46. Lateral chlamydospore on potato-dextrose agar.
Fig. 47. Lateral outgrowth as fig. 40, on Richards' agar.
Fig. 48. Lateral pyriform conidium on Richards' agar.
Fig. 49. Mycelium on the same agar.
Fig. 50. Broken young ascus showing thick wall, on the same medium.
Fig. 51. Adult ascus in a water mount, showing oil globules on potato-dextrose agar.




MOORE—POSADASIA CAPSULATA

## EXPLANATION OF PLATE

### PLATE 12

*Posadasia capsulata*

All figures drawn as correctly as possible with the aid of a camera lucida at a magnification of × 1440 and reduced to × 750, except fig. 71, which was drawn at a magnification of × 960 and reduced to × 510.

Fig. 52. Mycelium on Richards' agar.

Fig. 53. Thick-walled cell below surface of Sabouraud's agar, probably a young ascus.

Fig. 54. Mycelium on potato-dextrose agar, showing lateral young asci.

Fig. 55. Mycelium with hyphal swellings on wort agar.

Fig. 56. Young ascus with fine prickles on Sabouraud's agar.

Figs. 57–58. Asci with blunt tubercles on wort agar.

Fig. 59. Large spherical cell on nutrient agar mounted in lacto-phenol, showing internal vacuolation.

Fig. 60. Mycelium on potato-dextrose agar, showing asci, conidia, and clavate terminal cell.

Figs. 61–63. Types of asci on wort agar.

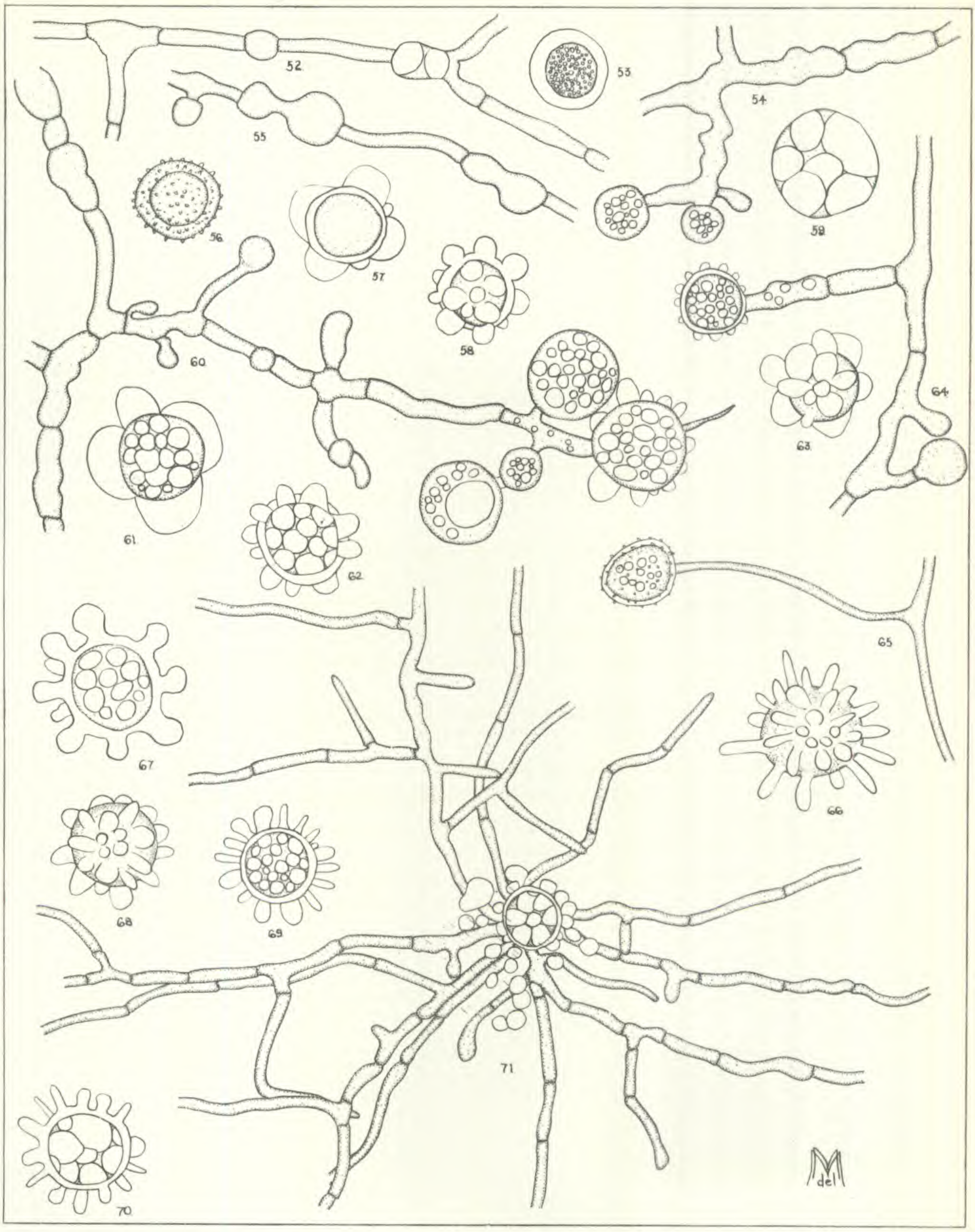
Figs. 64–65. Mycelium with asci on filaments, on potato-dextrose agar.

Figs. 66–68. Types of asci with different tubercles on wort agar.

Fig. 69. Ascus showing oil globules in a water mount from lactose agar.

Fig. 70. Ascus mounted in lacto-phenol, showing network of cell on nutrient agar.

Fig. 71. Ascus which had germinated in a gelatine slide culture, 10 days after inoculation.




MOORE—POSADASIA CAPSULATA

## EXPLANATION OF PLATE

### PLATE 13

*Posadasia pyriformis*

All figures drawn as correctly as possible with the aid of a camera lucida at a magnification of × 1440 and reduced to × 750.

Fig. 72. Group of conidia, and small round cells, probably ascospores, on Sabouraud's agar.

Fig. 73. Filament with a clavate terminal portion on Raulin's agar.

Fig. 74. Hypha on serum agar.

Fig. 75. Mycelium with swellings on wort agar.

Fig. 76. Peculiar type of lateral branching on Richards' agar.

Fig. 77. Racquet mycelium on potato-dextrose agar.

Fig. 78. Branching of hypha on glycerine agar.

Fig. 79. Intercalary chlamydospore on Sabouraud's agar.

Fig. 80. Mycelium on Richards' agar.

Fig. 81. Racquet formation on malt extract agar.

Figs. 82–83. Enlarged cells on wort agar.

Fig. 84. Mycelium on glycerine agar.

Fig. 85. Mycelium on corn-meal agar.

Fig. 86. Thick hypha on Richards' agar.

Fig. 87. Swollen intercalary cell on Raulin's agar.

Fig. 88. Mycelium on wort agar.

Fig. 89. Intercalary chlamydospores on Raulin's agar.

Figs. 90–91. Mycelium on wort agar.

Fig. 92. Lateral outgrowth, showing an intercalary chlamydospore on Richards' agar.

Fig. 93. Terminal clavate cells on Raulin's agar.

Fig. 94. Lateral pedicel with a terminal clavate cell on potato-dextrose agar.

Figs. 95–96. Chains of spherical cells on serum agar.

Fig. 97. Racquet mycelium on corn-meal agar.

Fig. 98. Terminal clavate cell, showing oil globules, on Raulin's agar.

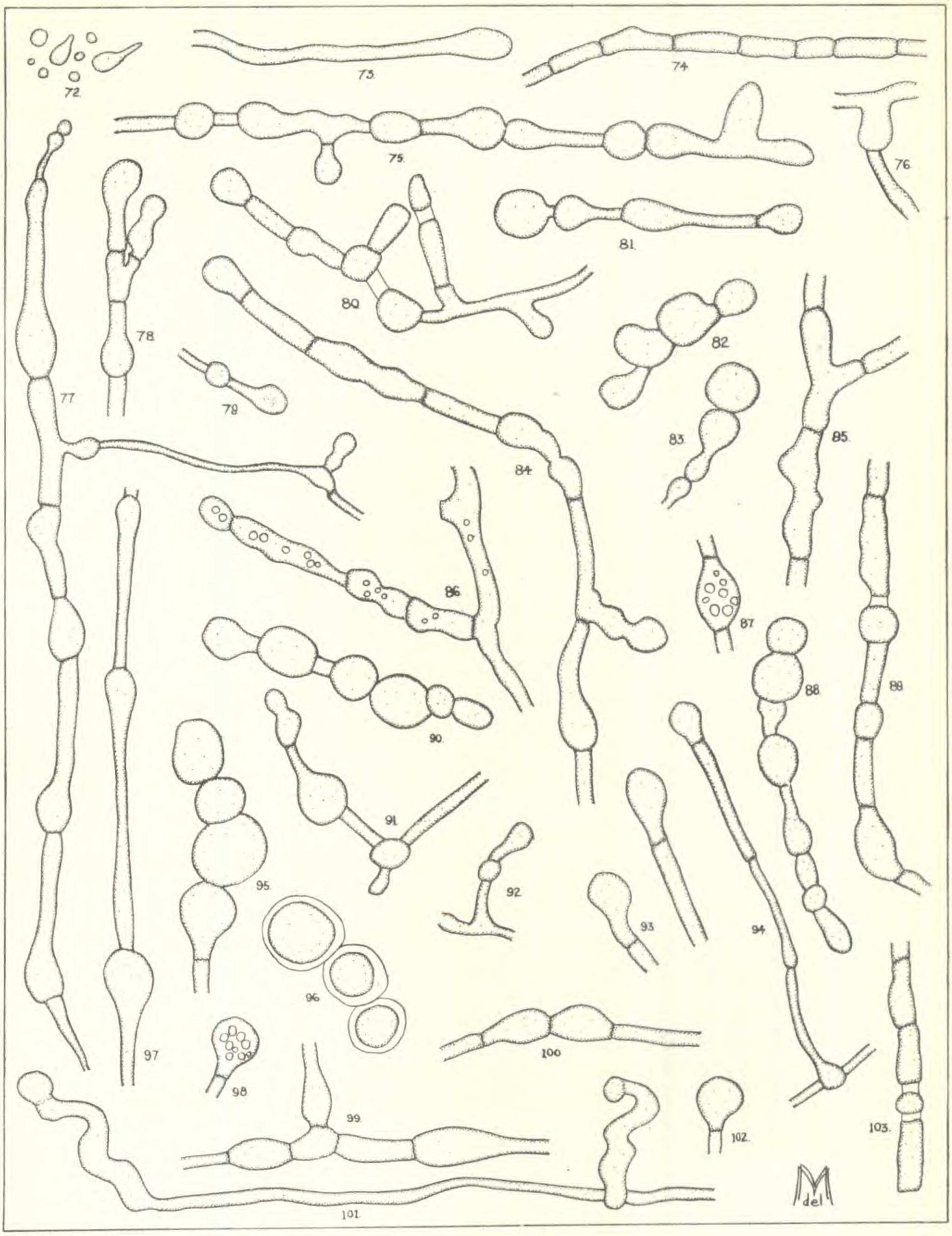
Fig. 99. Mycelium on Richards' agar.

Fig. 100. Hyphal swellings on glycerine agar.

Fig. 101. Mycelium showing snake-like processes which are presumptive asci bearers on Richards' agar.

Fig. 102. Terminal clavate cell on Raulin's agar.

Fig. 103. Mycelium showing intercalary chlamydospore on Raulin's agar.




MOORE—POSADASIA PYRIFORMIS

COCKAYNE, BOSTON

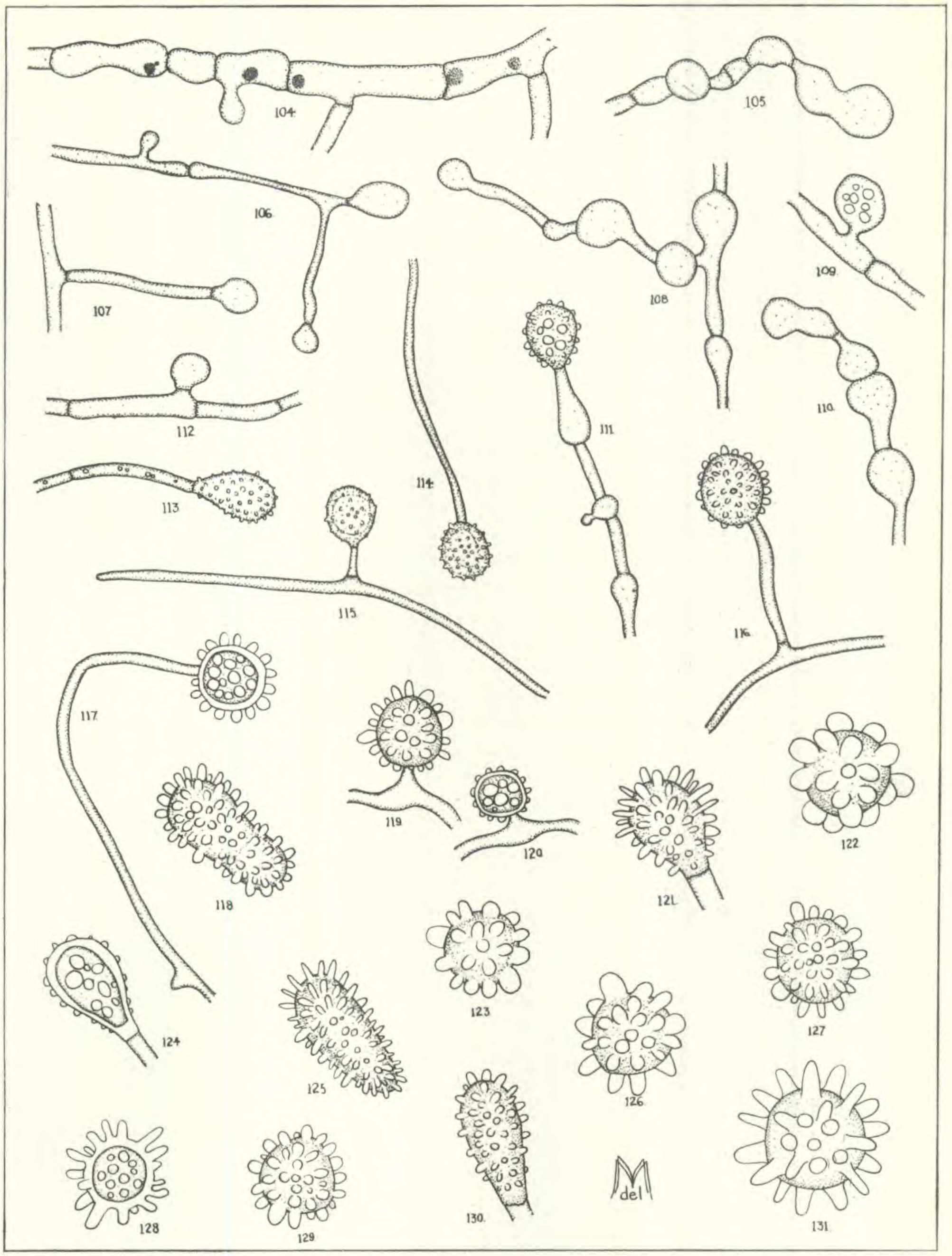
## Explanation of Plate

### PLATE 14

*Posadasia pyriformis*

All figures drawn as correctly as possible with the aid of a camera lucida at a magnification of × 1440 and reduced to × 750.

Fig. 104. Enlarged and thickened mycelium on corn-meal agar.
Fig. 105. Mycelium on wort agar.
Fig. 106. Mycelium showing lateral pedicellate clavate cell on Sabouraud's agar.
Fig. 107. Same as fig. 106, on corn-meal agar.
Fig. 108. Hyphal swellings on malt extract agar.
Fig. 109. Lateral outgrowth on potato-dextrose agar.
Fig. 110. Mycelium on malt extract agar.
Fig. 111. Terminal pyriform ascus on malt extract agar.
Fig. 112. Probably a conidium on Richards' agar.
Fig. 113. Terminal pyriform ascus on corn-meal agar.
Fig. 114. Same as fig. 113, on Sabouraud's agar.
Fig. 115. Lateral pedicellate ascus on corn-meal agar.
Fig. 116. Same as fig. 115, on the same medium.
Figs. 117–131. Various types of asci on different media.
Figs. 117, 119–120, 124, 126. On Richards' agar.
Figs. 118, 121, 125, 127, 129–130. On Raulin's agar.
Fig. 122. On glycerine agar.
Fig. 123. On Sabouraud's agar.
Fig. 128. On Czapek's agar.
Fig. 131. On potato-dextrose agar.




MOORE—POSADASIA PYRIFORMIS